44. (new) The method of claim 34 in which said liposomes are administered weekly.

45. (new) The method of claim 34 in which said liposomes are administered one dose per week for 4 to 16 weeks.

46. (new) The method of claim 34 in which said liposomes are administered one dose per week for 10 weeks.

47. (new) A method for treating a vascular disease or condition selected from the group consisting of atherosclerosis, hyperlipidemia and hypoalphalipoproteinemia in a human, comprising administering to a human in need thereof a pharmaceutically acceptable and a therapeutically effective amount of unilamellar liposomes comprising phospholipids having a size distribution, wherein the mean diameter of said liposomes is greater than about 95 nm, which liposomes remove more cholesterol from peripheral tissues than an equal amount of unilamellar liposomes comprising phospholipids having a mean diameter of 30 ± 7 nm as measured by calculating the difference between the mass of cholesterol removed from the circulation of mice injected with unilamellar liposomes having a mean diameter greater than about 95 nm and mice injected with unilamellar liposomes having a mean diameter of 30 ± 7 nm respectively.

REMARKS

The Applicants wish to express their appreciation to Examiner Kishore for the in-person interview courteously extended on January 30, 2003. During the interview, the proposed claims which define the liposome compositions used in the invention by their structural properties were discussed. The Applicants note with appreciation the Examiner's indication that such claims which define the liposomes used in the invention as a population characterized by a specific percentage of specifically sized liposomes falling within one standard deviation from the mean would be favorably considered.

The Claim Amendments

Claims 1-16 have been canceled without prejudice. The cancellation of claims 1-16 were made herein for the purpose of pursuing such canceled subject matter in related applications at the option of the Applicants and not for patentability. Thus, the Applicants expressly reserve the right to prosecute any unclaimed or canceled subject matter in the present application or in any related application. New claims 21-47 have been added.

In accordance with the Examiner's suggestions, the Applicants have used the term "treating" exclusively in all the claims, added a Markush group to clarify but not limit the term "vascular disease" and replaced the phrase "not bound to drug" with "free of drug." The examiner's suggestions are incorporated into new claims 21-47.

The new claims are fully supported by the specification and claims as originally filed and do not constitute new matter. Specifically, the new claims are supported by the specification at the following page and line numbers: Claims 21-22 and 34-35 are supported at p. 1, *ll.* 14-20; p. 2, *ll.* 16-18; p. 3, *ll.* 2-12; p. 6, *ll.* 6-7; p. 9, *ll.* 12-15; p. 10, *ll.* 10-17; p. 20, *ll.* 12-13; and p. 39, *ll.* 9-21 (disclosing the treatment of several vascular conditions including atherosclerosis, hyperlipidemia, and hypoalphalipoproteinemia); p. 6, *ll.* 18-22; p. 12, *ll.* 6-9 and original claim 1 (describing a method of administering to a human a pharmaceutically acceptable and therapeutically effective amount of unilamellar liposomes comprised of phospholipids); p. 78, *ll.* 7-11 (describing liposomes having mean diameters in the range of about 125 nm ± 30 nm which are expressed as the mean within plus or minus 1 standard deviation ["S.D."]); p. 13, *ll.* 12-13 and p. 68, *ll.* 14-16 (describing liposomes with diameters of about 100 nm); p. 25, *l.* 7 (describing liposomes having diameters of about 120 nm); p. 20, *l.* 7 (describing liposomes having diameters of about 125 nm); and p. 73, *ll.* 12-15 (describing liposomes having diameters in the range of about 100-150 nm). Claims 23 and 36 are supported at p. 20, *l.* 3 (indicating that the liposomes are not bound to or are otherwise free of drug). Claims 24-27 and 37-40 are supported at p. 54, *ll.* 12-24 and p. 76, *ll.* 1-4 (disclosing doses of about 10-1600 mg/kg, 300 mg/kg, 0.1-1.5 gm/kg, and 0.28-0.42 gm/kg). Claims 28-33 and 41-46 are supported at p. 13, *ll.* 10-11 and p. 75, *ll.* 21-24 (disclosing the administration of liposomes once, more than once, in repeated doses, weekly, for 4-16 weeks, and for 10 weeks). Claim 47 is supported by the specification at p. 78, *ll.* 3-20 and p. 81, *l.* 5 to p. 87, *l.* 2.

Finally, the specification has been amended to update the priority information for the present application in accordance with and reflected in the application data sheet. The specification has also been amended to correct some minor typographical errors in the second sentence of page 76 which are fully supported by prior U.S. Application No. 08/206,415 to which the instant application claims benefit.

The Claimed Invention

The new claims define the liposome preparation used in the claimed methods as a "pharmaceutically acceptable and a therapeutically effective amount of unilamellar phospholipid liposomes wherein at least 68% of the liposomes have a mean diameter of 125 ± 30 nm." This is supported by the specification which describes the preparation and use of a population of liposomes having a diameter of 125 nm plus or minus 30 nm as measured by QELS (Quasi-Electric-Light-Scattering) analysis, utilizing a Nicomp Model 370 submicron laser particle sizer (Pacific Scientific, MD) equipped with a 5-mW He-Ne Laser (*see* specification at p. 78, *ll.* 3-11). As explained in the specification, the Nicomp QELS system used to characterize the liposome population analyzes fluctuations in light-scattering intensities due to liposome diffusion in solution. *Id.* The measured diffusion coefficient is

used to obtain the average hydrodynamic radius (*see* specification at p. 78, *ll.* 5-6) and the mean diameter of liposomes is expressed as the mean plus or minus 1 standard deviation (125 ± 30 nm) (*see* specification at p. 78, *ll.* 3-11), arrived at using a Gaussian analysis (*see, The Nicomp 370 Model Submicron Particle Sizer User Manual* at pp. 24-25, entitled, "The Simplest Approach to Size Distributions: Gaussian Analysis," attached hereto as Exhibit C).[1]

This analysis uses well-known and accepted mathematical principles to characterize the size distribution of a population, which is expressed as a Gaussian distribution, also known as a "normal" or "bell-shaped" distribution (*see An Introduction to Statistics-Lesson 6: The Bell Shaped, Normal, Gaussian Distribution*, attached hereto as Exhibit D). In a Gaussian, normal, or bell-shaped distribution 68% of the data elements are within one standard deviation of the mean, 95% are within two standard deviations, and 99.7% are within three standard deviations (see Exhibit D, at p. 2, "The Empirical Rule," often stated simply as 68-95-99.7). Thus, the Applicants are claiming the use of a population of liposomes with a very specific Gaussian distribution -- a specific population of liposomes defined by a bell-shaped curve, in which 68% of the population falls within one standard deviation (30 nm) from the mean (125 nm).

The use of a liposome preparation defined by the claims is not disclosed by the prior art and has properties which are surprising and unexpected in view of the teachings of the prior art. Specifically, a population of liposomes wherein at least 68% of the liposomes have a mean diameter of 125 ± 30 nm will surprisingly and unexpectedly mobilize more cholesterol and will control or prevent a rise in LDL and/or esterified cholesterol in contrast to the liposomes disclosed in the prior art (*see* specification at p. 25, *ll.* 1-22; p. 28, *ll.* 1-9; p. 83, *l.* 1 to p. 84, *l.* 2; and Figures 3, 4, 10-12, & 35). *See also* Exhibit E, Rodrigueza et al., *Large Versus Small Unilamellar Vesicles Mediate Reverse Cholesterol Transport In Vivo Into Two Distinct Hepatic Metabolic Pools: Implications For the Treatment of Atherosclerosis,* 17 ARTERIOSCLER. THROMB. VASC. BIOL. (10): 2132-39, 2134 (1997). The prior art describes the use of smaller liposomes which is associated with significant side effects such as increases in plasma LDL and/or esterified cholesterol levels that can occur after the administration of such liposomes. *See* Figure 2(A) of Williams 1986 which shows that 4 hours after infusion with SUVs (small unilamellar vesicles) there was a dramatic increase in LDL and Figure 5 of Williams 1986 which shows an increase in LDL (peak P1) after SUV treatment. In contrast, LUVs (large unilamellar vesicles) having the claimed Gaussian distribution do not substantially increase LDL or esterified cholesterol and mobilize more cholesterol than SUVs. *See* specification at p. 25, *ll.* 1-22; p. 28, *ll.* 1-9; p. 83, *l.* 1 to p.

[1] The Nicomp Model 370 automatically selects the appropriate statistical/mathematical procedures to analyze the "raw data" generated by the system (see Exhibit C, p. 24). The Gaussian Analysis, coined as the "simplest approach to size distributions" is the one typically obtained for emulsions prepared by a variety of processes, including sonication, homogenization and microfluidization (see Exhibit C, p. 25).

84, *l.* 2; and Figures 3, 4, 10-12, & 35); Figure 2 arrow 2 in Rodrigueza et al., *The Influence of Size and Composition On the Cholesterol Mobilizing Properties Of Liposomes In Vivo*, 1153 BIOCHIMICA BIOPHYSICA ACTA 9-19 (July 1993) (showing no increase in LDL cholesterol after LUV treatment); and Rodrigueza et al., *Cholesterol Mobilization And Regression of Atheroma In Cholesterol-Fed Rabbits Induced By Large Unilamellar Vesicles*, 1368 BIOCHIMICA BIOPHYSICA ACTA 306-320, 312 (col. 2, *ll.* 32-34) (1998) (stating that no changes in esterified cholesterol [CE] plasma concentrations were detected following LUV injections).

Thus, in contrast to the "oceans of liposomes" (as expressed by the Examiner) that may be disclosed by the prior art,[2] the Applicants are claiming the specific use of a specific population defined by their structure and possessing the surprising and unexpected properties of: (1) better cholesterol mobilization and/or, (2) cholesterol mobilization without a substantial increase in LDL or esterified cholesterol levels. The claimed method was unknown prior to the present invention. For the Examiner's convenience, the Applicants submit herewith a chart summarizing the differences between the claimed invention and the prior art cited by the Examiner in this and related applications, (attached hereto as Exhibit G).

1. <u>The Claims are Patentable Over Hager Under 35 U.S.C. § 102</u>

Claims 1-2, 4-9 and 11-14 were rejected under 35 U.S.C. § 102(b) as being anticipated by EP 0 470 437. According to the Examiner EP 0 470 437 ("Hager") teaches unilamellar liposomes having a mean diameter of 100 nm containing phosphatidylcholine for the treatment of atherosclerosis and increased fat values which implicitly includes dislipidemia (noting p. 7 of the translation and columns 5-7 of the English equivalent). This rejection is moot since claims 1-16 have been canceled. Nevertheless, Applicants demonstrate below that the newly presented claims are not anticipated by this reference.

Hager does not teach a pharmaceutically acceptable and a therapeutically effective amount of unilamellar phospholipid liposomes wherein at least 68% of the liposomes have a mean diameter of 125 ± 30 nm. Anticipation requires identity of the invention. *Akzo N.V. v. International Trade Comm'n*, 808 F2d 1471, 1479-81 (Fed. Cir. 1986). Hager does not disclose each and every element of the claimed invention. Thus, Hager cannot anticipate the claims. At best, Hager describes ranges that may overlap with the range in Applicant's claims but Hager does not disclose a blank liposome suitable for human use that has the claimed Gaussian distribution with sufficient specificity to constitute anticipation.[3] For example in *Akzo N.V. v. International Trade Comm'n*, 808 F2d 1471,

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[2] Applicants respectfully point out that much of the art that the Examiner is referring to relates to loaded liposomes or liposomes carrying active agents or drugs.

[3] Although Example 3 of Hager discloses liposomes of 129 nm, these liposomes are bound to propidium iodide (a DNA marker that is a known "mutagen" and "irritant,") which is not intended to be

1479-81 (Fed. Cir. 1986) the court held that claims to a process of making aramid fibers using a 98% solution of sulfuric acid were not anticipated by a reference which disclosed using sulfuric acid solution but which did not disclose using a 98% concentrated sulfuric acid solution. *See also* MPEP 2131.03, entitled "Anticipation of Ranges" ("In order to anticipate the claims, the claimed subject matter must be disclosed with sufficient specificity to constitute an anticipation under the statute.").

Thus, because the liposomes of the presently claimed method requires that the liposomes are: (a) administered to humans, (b) "pharmaceutically acceptable" (free of propidium iodide, *see* Exhibit F), and (c) required to have a particular Gaussian distribution, Hager does not teach each and every element of the claims, either expressly or inherently, and therefore does not anticipate the claims under 35 U.S.C. § 102(b).

2. <u>The Rejections Under 35 U.S.C. § 103 Should Be Withdrawn</u>

A. The New Claims are patentable over Hager

Claims 1-9 and 11-14 were rejected under 35 U.S.C. § 103(a) as being obvious over Hager. This rejection is moot in view of the cancellation of claims 1-16. The new claims define subject matter that is not obvious over the art for the reasons detailed below.

A finding of obviousness requires that the prior art both suggest the invention and provide one of ordinary skill with a reasonable expectation of success. *In re O'Farrell* 853 F2d 894, 903, 7 USPQ2d 1673 (Fed. Cir. 1988). Secondary considerations such as unexpected results must be considered if present. *Stratoflex, Inc. v. Aeroquip Cor*p., 713 F.2d 1530, 1538-39, 218 USPQ 871, 879 (Fed. Cir. 1983); *In re Merck & Co., Inc.*, 800 F.2d 1091, 1096, 231 USPQ 375, 378 (Fed. Cir. 1986). Here, the Applicant's method of using liposome populations having the claimed Gaussian distribution is not suggested by the prior art and achieves unexpected results.

Applicant's unexpected results lie in the discovery that liposomes having the claimed size distribution wherein at least 68% of the liposomes have a mean diameter of 125 ± 30 nm: (1) do not substantially raise LDL or esterified cholesterol levels; and (2) mobilize more cholesterol from peripheral tissues (such as atherosclerotic plaques) than an equivalent amount (per weight) of liposomes having a different Gaussian distribution with smaller liposomes (*see* specification at p. 25, *ll.* 1-22; p. 28, *ll.* 1-9; p. 83, *l.* 1 to p. 84, *l.* 2; and Figures 3, 4, 10-12, & 35). Liposomes with a size distribution having a smaller mean diameter can cause a substantial rise in LDL or esterified cholesterol levels (bad cholesterol) which is associated with the development and progression of atherosclerosis; thereby destroying the usefulness of the liposomes for clinical use. *Id.*; s*ee also* Applicants post-filing

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administered to a human and not "pharmaceutically acceptable" as required by the claims (*see* Exhibit F, a copy of a Material Safety Data Sheet on propidium iodide from Sigma-Aldrich Corporation; *see also* Aldrich Catalog, p. 1432, 1998-1999). Furthermore, Example 3 does not disclose the Gaussian distribution as claimed.

peer reviewed article, Rodrigueza et al., *Large Versus Small Unilamellar Vesicles Mediate Reverse Cholesterol Transport In Vivo Into Two Distinct Hepatic Metabolic Pools: Implications For the Treatment of Atherosclerosis,* 17 ARTERIOSCLER. THROMB. VASC. BIOL. (10): 2132-39, 2134 (1997) (large unilamellar liposomes (123 ± 35 nm) were more efficient in mobilizing unesterified cholesterol than small unilamellar liposomes (34 ± 30 nm), and animals treated with small unilamellar liposomes developed elevated concentrations of esterified cholesterol in contrast to animals treated with liposomes greater than about 123 ± 35 nm which showed no change in esterified cholesterol levels).

Prior to the present invention, only small liposomes (*e.g.*, 21-60 nm) were thought to be useful for the treatment of atherosclerosis. For example, it was generally assumed that the smaller the liposome size, the greater the circulation half-life, and therefore the more cholesterol mobilized (Gregoriadis and Senior, LIFE SCI. 113:183-192 (1986)). It was also expected that smaller liposomes would produce a greater number of HDL-like particles, thus promoting efflux of sterol from peripheral tissues (*see* p. 11, *ll.* 7-20 of the specification citing several prior publications related to this subject). Accordingly, the view prior to the present invention was that small liposomes (*i.e.*, 21-60 nm) were better than larger ones (*i.e.* 123 ± 35 nm). This is a clear teaching away from the claimed invention which contradicts any contention of obviousness.

Hager teaches the same when considered in view of the prior art as a whole. In fact, Hager teaches using liposomes with a mean diameter as low as 50 nm. Since the prior art teaches away from using a population of liposomes falling within the claimed Gaussian distribution, the claimed invention is not obvious.

In sum, the claims are not obvious over Hager because: (1) the prior art does not suggest using pharmaceutically acceptable liposomes having the claimed Gaussian distribution in humans; (2) the Applicants have unexpected results showing that liposomes falling within the claimed Gaussian distribution do not substantially raise LDL or esterified cholesterol levels and mobilize more cholesterol from peripheral tissues than liposomes falling outside the claimed Gaussian distribution; (3) the prior art teaches that liposomes falling outside the claimed Gaussian distribution (*i.e.*, small liposomes of 21-60 nm) are better; and (4) Hager teaches nothing different than the prior art when the prior art is considered as a whole.

B. The New Claims Are Patentable over Williams by Itself or in Combination with Hager

Claims 1-15 are rejected under 35 USC 103(a) as being unpatentable over Williams (BBA, 875, pp. 183-94, 1986) by itself or in combination with Hager. This rejection is moot in view of the cancellation of claims 1-16. The new claims define subject matter that is not obvious over Williams by itself or in combination with Hager for the reasons detailed below.

Williams 1986 does not cure the deficiencies of Hager since Williams also does not suggest using a population of liposomes falling within the claimed Gaussian distribution. Williams 1986 describes the preparation of SUVs[4] and the uptake of endogenous cholesterol when the SUVs are administered to dogs. The data presented in Williams 1986 shows that 4 hours after infusion with SUVs a dramatic increase in LDL occurs as a result of this treatment (*see* Williams 1986, Fig. 2A, the peak labeled "P1" [LDL] at four hours ["t = 4h"]).

Thus, Williams 1986 not only discloses liposomes that are smaller than those presently claimed but the reference actually teaches away from the presently claimed invention because it shows an increase in esterified cholesterol and LDL after administration.

Accordingly, the rejection under 35 USC 103(a) as being unpatentable over Williams 1986 (BBA, 875, pp. 183-94, 1986) by itself or in combination with Hager should be withdrawn. whole.

C. Claim 16 is Patentable over Hager or Williams in Further View of Allen

Claim 16 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Hager or Williams (BBA, 875, pp. 183-94, 1986) in further view of Allen (J. Liposome Research, 1992). The Applicants respectfully note that claim 16 has been canceled and therefore the rejection is moot.

3. The Rejection Under 35 U.S.C. §112 Second Paragraph, Should Be Withdrawn

The rejection under 35 U.S.C. § 112, second paragraph, is obviated by the new claims. For example, the terms "large," "small cholesterol acceptor," "a drug which increases HDL concentrations," "phosphatidylcholine mixture," "amphiphilic compound," and "lipid binding proteins" do not appear in new claims 21-47. Furthermore, the Applicants invite the Examiner's attention to p. 68, *ll.* 21-22, indicating that the term "small" generally relates to a size of significantly less than 100 nm (such as 30 nm, p. 11, *ll.* 5-9). Accordingly, the rejection under 35 U.S.C. §112, second paragraph, should be withdrawn.

[4] In Williams 1986, the phospholipid dispersion was prepared using ultrasonic irradiation (with a titanium probe) for a total of 40 minutes, followed by ultracentrifugation for 1 hour at 100,000 x g to remove fragments of titanium shed by the sonicator probe (*see* Williams 1986 at p. 184, col. 2, last paragraph). This method results in the production of SUVs -- a result confirmed by the data in Williams 1986, Fig. 5 which shows that Williams' SUVs co-elute with the LDL fraction ("P1"), which is known to be 30 nm.

4. **The Obviousness-Type Double Patenting Rejection Should Be Withdrawn**

The rejection under the judicially created doctrine of obvious-type double patenting is obviated in view of the differences between the new claims and the claims of U.S. Patent Nos. 5,746,223 and 6,312,719. In the alternative, the Applicants respectfully request that the Examiner hold this rejection in abeyance until the new claims are otherwise deemed allowable at which time the Applicants will file a terminal disclaimer if appropriate based on the final version of the claims allowed.

CONCLUSION

Entry of the foregoing amendments and remarks is respectfully requested. No fee is believed to be due with this Reply other than the fee for a Petition for Extension of Time. However, if any other fee is required, please charge the fee to Pennie & Edmonds LLP Deposit Account No. 16-1150. If any issues remain, the Examiner is requested to telephone the undersigned at (212) 790-9090.

Respectfully submitted,

Date March 20, 2003

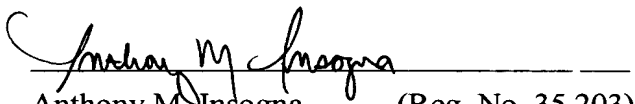

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Exhibit A
U.S. Application No.: 09/924,222
Marked Up Version of Amendments in the Specification

Page 1, lines 4-11 of the Specification

[This application is a continuation-in-part application, and claims priority to copending United States patent application serial no. 09/071,974, filed May 4, 1998, which claims priority to United States patent application serial no. 08/728,766, filed October 11, 1996, now U.S. Patent No. 5,746,223, which further claims the benefit of priority to United States provisional patent application serial number 60/005,090 filed by Kevin Jon Williams on October 11, 1995, entitled "METHOD OF FORCING THE REVERSE TRANSPORT OF CHOLESTEROL FROM PERIPHERAL TISSUES TO THE LIVER IN VIVO WHILE CONTROLLING PLASMA LDL AND COMPOSITIONS THEREFOR."] This application is a continuation-in-part of United States Patent Application No. 09/332,336, filed May 28, 1999, now U.S. Patent No. 6,312,719, which is a continuation of United States Patent Application No. 09/175,553 filed October 20, 1998, now U.S. Patent No. 6,139,871, which is a continuation of United States Patent Application No. 08/507,170 filed July 26, 1995, abandoned, which is a continuation of United States Patent Application No. 08/206,415 filed March 4, 1994, abandoned, each of which is incorporated herein by reference in their entirety. This application is also a continuation-in-part of United States Patent Application No. 09/071,974, filed May 4, 1998, which is a divisional of United States Patent Application No. 08/728,766, filed October 11, 1996, now U.S. Patent No. 5,746,223, which claims the benefit of priority to United States Provisional Patent Application No. 60/005,090 filed October 11, 1995, each of which is incorporated herein by reference in their entirety.

Second Full Sentence on Page 76, lines 2-4 of the Specification

Humans will generally be treated with about 0.1-1.5 gm[.] of liposomes/kg body weight, usually about 0.2-0.75 [gin] gm/kg, and most usually about 0.28[Z]-0.42 gm/kg.